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(54) Anorexigenic tripeptides, process for the preparation thereof and pharmaceutical compositions containing them

(57) Tripeptides having anorexigenic activity of the formula (I):

X—Y—Pro-OH (I)

in which

X is Glp, Kic, Tca;

Y is Gly, Ala, Val, Ile, Pro, Abu, Nva, Nle, Ada, Cha, Phe, Trp, Tyr, Ser, Thr, Met, Gln, Lys, Arg, Leu,and acid addition salts and complexes thereof.

The tripeptides are prepared by coupling proline or a carboxyl-protected derivative thereof with the amino acids X and Y or protected derivatives thereof by known methods of peptide chemistry, with the consequential removal of protecting group(s) if present.

There are also provided pharmaceutical compositions having anorexigenic activity, which comprise, as the active ingredient, tripeptides of the formula (I), pharmaceutically acceptable acid addition salts or complexes thereof.

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SPECIFICATION

Anorexigenic tripeptides, process for the preparation thereof and pharmaceutical compositions containing them

This invention relates to new tripeptides having anorexigenic activity, a process for their preparation and pharmaceutical compositions containing them.

More particularly, the invention provides new tripeptides of the formula (I):

X-Y-Pro-OH (I)

in which

X is Glp, Kic or Tca;

Y is Gly, Ala, Val, Ile, Pro, Abu, Nva, Nle, Ada, Cha, Phe, Trp, Tyr, Ser, Thr, Met, Gln, Lys, Arg or Leu,

and acid addition salts and complexes thereof.

According to another aspect of the invention, there is provided a process for the preparation of the tripeptides of the formula (I) and acid addition salts and complexes thereof.

The invention further provides new pharmaceutical compositions having anorexigenic activity, which comprise compounds of the formula (I) or pharmaceutically acceptable salts or complexes thereof as an active ingredient.

Reichelt et al. [Neuroscience *3*, 1207 (1978)] isolated a Glp-His-Gly-OH tripeptide from the urine of patients suffering from anorexia nervosa. The tripeptide showed anorexigenic activity in animal tests. This experimental result was an important contribution to the earlier assumption that certain peptides may play an essential role in the so far unknown mechanism of the development of the sensation of hunger and repletion, respectively. Taking into account the structural similarities between the above tripeptide and TRH (thyrotropin-releasing hormone) (Glp-His-Pro-NH_2) it may well be assumed that both peptides are formed from the same precursor molecule.

We have found that a precondition of the anorexigenic activity is the presence of a free carboxyl group at the C-terminal moiety since the compounds containing a C-terminal carboxamido group showed no anorexigenic activity. We have further found that the presence of histidine in the centre of the molecule is not essential. The compounds in which the central amino acid has an aliphatic side-chain and the C-terminal amino acid is a free carboxyl-containing proline proved to be the most effective.

The compounds of the formula (I):

X-Y-Pro-OH (I)

in which X and Y have the same meaning as defined above, can be prepared by any method conventionally used in the chemistry of peptides. Preferably, the amino acids X and Y or protected derivatives thereof are coupled to the proline or a carboxyl-protected derivative thereof by a conventional technique of peptide chemistry, and, if the tripeptides are obtained in a protected form the protecting groups are eliminated therefrom in a known manner.

According to a preferred embodiment of this process, proline methyl ester is acylated by a protected amino acid tert.-butyloxycarbonyl-Y-OH, the dipeptide methyl ester obtained is saponified with a dioxanic sodium hydroxide solution and the tert-butyloxycarbonyl protecting group is eliminated by treatment with hydrochloric acid/ethyl acetate. The Y-Pro-CH.HCl dipeptide salt is then acylated with benzyloxycarbonyl-pyroglutamic acid pentafluorophenyl ester, and the benzyloxy group is eliminated from the protected tripeptide by catalytic hydrogenolysis.

The compounds of the formula (I) can be purified by conventional purification techniques, preferably column chromatography or by precipitation from a suitable solvent. The compounds are obtained as lyophilized powders or solid powders, which can be formulated into various pharmaceutical preparations.

The anorexigenic activity of the compounds of the formula (I) was tested on rats. The food consumption of test animals starved for 96 hours was measured one and five hours after the intra-cerebroventicular (icv.) administration of a physiological saline solution (control) and the test materials, respectively. The corresponding data for certain compounds according to the invention, TRH and the tripeptide Glp-His-Gly-OH are set forth in the following Table 1.

Table 1

	Dose (μg)	*Time of food administration after injection (hours)*	*Route of administration*	*Food consumption (g) in 24 hours of rats starved for 96 hours*
Control	—	5	icv.	22.16±0.88
Glp-His-Pro-NH_2	300	1	icv.	19.81±1.52
	300	5	icv.	17.88±2.94
Glp-His-Gly-OH	300	1	icv.	19.33±1.08
	300	5	icv.	18.38±1.80
Glp-Leu-Pro-OH	300	1	icv.	6.10±1.99
		5	icv.	9.23±0.92
Glp-Abu-Pro-OH	300	5	icv.	8.31±1.50

The data clearly show that neither THR nor Glp-His-Gly-OH reduced substantially the food consumption of the test animals under the given test conditions, unlike the tested representatives of the compounds of the formula (I) which results in a significant decrease of food uptake.

The peptides according to the invention, their salts and complexes are used in the therapy as pharmaceutical compositions. These compositions comprise the compounds according to the invention in association with conventional, pharmaceutically acceptable inorganic or organic carriers or excipients suitable for enteral or parenteral administration. Solid lyophilizates may also be prepared, in which materials inert to the peptides, e.g. carbohydrates, are generally used as carriers. Further pharmaceutical formulations include dilute or concentrated suspensions and emulsions which may also contain various preserving and stabilizing additives.

The invention will be illustrated in greater detail in two general Examples which are given for illustration and not limitation of our invention. The compounds prepared by one of the illustrated methods together with their physical constants are given in Table 2.

The abbreviations used in the Examples and throughout the specification are internationally accepted designations [J. Biol. Chem. *247*, 977 (1972)]. Further abbreviations:

Abu=L-2-aminobutyric acid;
Ada=L-2-aminodecanic acid;
Cha=L-cyclohexylalanine;
Klc=L-2-keto-imidazolidine-4-carboxylic acid;
Tca=L-thiazolidine-4-carboxylic acid.

Evaporation was always carried out in a Büchi Rotavapor "R" equipment. Melting points were determined in an apparatus by Dr. Tottoli (Büchi). The thin-layer chromatograms were prepared on "Kieselgel G" (Merck) silicagel phases according to Stahl. For preparing the chromatograms the following solvent mixtures were used:

(1) 3:2 mixture of ethyl acetate and PAW
(2) 1:1 mixture of ethyl acetate and PAW
(3) 3:1:1 mixture of *n*-butanol, acetic acid and water PAW=20:6:11 mixture of pyridine, acetic acid and water.

The thin layer chromatograms were developed by ninhydrin or by a tolidine/potassium iodide spray, after chlorination.

Certain compounds were purified on a column filled with "Kieselgel G" silica gel having a grain size of 0.062 to 0.2 mm.

Example 1

L-Pyroglutamyl-L-leucyl-L-proline (method "A")

Step 1

L-Leucyl-L-proline.HCl

5.5 g (33 mmoles) of Pro-OMe.HCl are dissolved in 80 ml of dichloromethane, the solution is cooled to 0°C and 4.52 ml (33 mmoles) of triethyl amine and subsequently 6.93 g (30 mmoles) of Boc-Leu-OH are added with stirring. As soon as the reactants are dissolved, a solution of 6.18 g (30 mmoles) of dicyclohexyl carbodiimide in 40 ml of dichloromethane is added, whereupon the reaction mixture is stirred at 0°C for 2 hours and the dicyclohexyl carbodiimide is filtered off. The filtrate is

shaken with three 30-ml portions of a 1 N hydrochloric acid solution, three 30-ml portions of a 1 N sodium hydrogen carbonate solution and 30 ml of water. The dichloromethane solution is dried with anhydrous sodium sulphate and evaporated. 9.7 g of Boc-Leu-Pro-OMe are obtained as an oily product. The product is dissolved in 43 ml of dioxane, and 43 ml of a 1 N sodium hydroxide solution are added with stirring. The reaction mixture is stirred at room temperature for one hour and neutralized with a 1 N hydrochloric acid solution. Dioxane is distilled off and the pH of the aqueous solution obtained is adjusted to 2 by adding 60 ml of chloroform and concentrated sulphuric acid. After separation the aqueous solution is shaken with a further two 30-ml portions of chloroform. The combined chloroform solution is shaken with water and dried over anhydrous sodium sulphate. The solvent is eliminated, the residual 9 g of Boc-Leu-Pro-OH are dissolved in 25 ml of ethyl acetate and 25 ml of a 5 N solution of hydrochloric acid in ethyl acetate are added to the solution. The reaction mixture is allowed to stand at room temperature for one hour, diluted with ether and the precipitated substance is filtered off, washed with ether and dried. 6.48 g (81.5% related to Boc-Leu-OH) of H-Leu-Pro-OH.HCl are obtained. The product is a chromatographically uniform, white, hydroscopic material. R_f^2=0.20; R_f^3=0.41.

Step 2

Benzyloxycarbonyl-L-pyroglutamyl-L-leucyl-L-proline

2.09 g (7.87 mmoles) of H-Leu-Pro-OH. HCl, 2.2 ml of triethyl amine and 3.38 g (7.87 mmoles) of benzyloxycarbonyl-L-pyroglutamic acid pentafluorophenyl ester are dissolved in 50 ml of chloroform. After standing for 10 minutes 1.1 ml (7.87 mmoles) of triethyl amine are added and the solution is allowed to stand overnight at room temperature. Thereafter, the reaction mixture is shaken with three 10-ml portions of a 1 N hydrochloric acid solution and two 10-ml portions of water, dried with anhydrous sodium sulphate and evaporated. Crystallization of the oily evaporation residue from ether affords 3.15 g of a crude product which is then recrystallized from 10 ml of ethyl acetate. 2.7 g (73.5%) of chromatographically uniform Z-Glp-Leu-Pro-OH are obtained, melting at 98 to 100°C.

Step 3

4.13 g (8.7 mmoles) of Z-Glp-Leu-Pro-OH are dissolved in 80 ml of ethanol, 0.8 g of a 10% palladium-on-charcoal catalyst are added and hydrogen gas is bubbled through the solution for one hour. The catalyst is filtered off, the filtrate is evaporated and the amorphous residue is triturated with ether and filtered off. 2.8 g of a crude product are obtained, which are then dissolved in 20 ml of water, decoloured with charcoal and the solution is lyophilized. 2.6 g (91%) of amorphous, chromatographically uniform Glp-Leu-Pro-OH are obtained. R_f^1=0.33.

Example 2

L-Pyroglutamyl-glicyl-L-proline (method "B")

Step 1

Glicyl-L-proline

5.33 g (22 mmoles) of H-Pro-OBzl-HCl and 4.2 g (20 mmoles) of Z-Gly-OH are dissolved in 50 ml of dichloromethane, 3.08 ml of triethyl amine are added to the solution, it is cooled at 0°C and a solution of 4.12 g (20 mmoles) of dicyclohexyl carbodiimide in 30 ml of dichloromethane is added. The reaction mixture is then stirred for one hour at 0°C and kept in a refrigerator overnight. Dicyclohexyl carbodiimide is filtered off and the filtrate is extracted with two 30-ml portions of a 1 N hydrochloric acid solution, two 30-ml portions of a 1 N sodium hydrogen carbonate solution and 30 ml of water. The dichloromethane solution is dried with anhydrous sodium sulphate and evaporated. 8.5 g of Z-Gly-Pro-OBzl are obtained as an oily substance, which is dissolved in 170 ml of ethanol, 1.5 g of a 10% palladium-on charcoal catalyst are added and hydrogen is bubbled through the solution. When the reaction is complete, the reaction mixture is diluted with 100 ml of water, the catalyst is filtered off, washed with water and the filtrate is evaporated. 2.12 g (62% related to Z-Gly-OH) of H-Gly-Pro-OH are obtained as a chromatographically uniform product. R_f^3=0.19.

Step 2

Benzyloxycarbonyl-L-pyroglutamyl-glycyl-L-proline

2.15 g (5 mmoles) of Z-Glp-OPfp and 0.86 g (5 mmoles) of H-Gly-Pro-OH are suspended in 20 ml of dimethyl formamide. 0.7 ml of triethyl amine are added to the suspension. After one hour the reaction mixture is evaporated, the residue is dissolved in 30 ml of chloroform and the solution is shaken with two 7-ml portions of a 1 N hydrochloric acid solution, two 7-ml portions of a 1 N sodium hydrogen carbonate solution and water. Upon drying the solution is evaporated. The oily residue is crystallized from ether, filtered off and the crude product, weighing 1.4 g, is recrystallized from a mixture of ethanol and ethyl acetate. 1.23 g (53%) of Z-Glp-Gly-Pro-OH are obtained as a chromatographically uniform product.

Step 3

0.35 g (0.84 mmoles) of Z-Glp-Gly-Pro-OH are dissolved in 30 ml of ethanol, 0.05 g of a 10% palladium-on-charcoal catalyst are added and hydrogen gas is bubbled through the solution. The catalyst is filtered off, the filtrate is evaporated and the residue is triturated with ether and filtered. 0.22 g of the title compound is obtained. The product is treated with ethyl acetate and filtered to yield 0.17 g (71.5%) of a chromatographically uniform, amorphous product. R_f^2=0.06; R_f^3=0.25.

The new compounds prepared according to any of the above methods are set forth in the following Table 2.

Table 2

Tripeptides	*Method*	*Melting point [°C]*	R_f^1	R_f^2	R_f^3
Glp-Ala-Pro-OH	A	234—236 (decomp.)		0.12	0.31
Glp-Val-Pro-OH	A	amorphous	0.20		
Glp-Ile-Pro-OH	A	amorphous	0.26		
Glp-Pro-Pro-OH	A	188—191 (decomp.)		0.07	0.25
Glp-Abu-Pro-OH	A	248—250 (decomp.)	0.11		
Glp-Nva-Pro-OH	A	183—186	0.19		
Glp-Nle-Pro-OH	A	amorphous	0.26		
Glp-Ada-Pro-OH	A	amorphous	0.35		
Glp-Cha-Pro-OH	A	amorphous	0.30		
Glp-Phe-Pro-OH	A	amorphous	0.22		
Glp-Trp-Pro-OH	A	204—206 (decomp.)	0.27		
Glp-Tyr-Pro-OH[a]	A	132—134 (decomp.)	0.22		
Glp-Ser-Pro-OH[b]	A	144—146 (decomp.)		0.07	0.25
Glp-Thr-Pro-OH[c]	A	179—181 (decomp.)		0.12	0.31
Glp-Met-Pro-OH[d]	A	amorphous	0.22		
Glp-Gln-Pro-OH[e]	A	amorphous		0.07	0.23
Glp-Lys-Pro-OH[f]	A	165—167 (decomp.)			0.16
Glp-Arg-Pro-OH[g]	A	297—298 (decomp.)			0.17
Glp-Gly-Pro-OH	B	amorphous		0.06	0.25
Klc-Leu-Pro-OH	A	amorphous		0.37	
Tca-Leu-Pro-OH[h]	A	157—160	0.48		

[a]Using Try(Bu^t)
[b]Using Ser(Bzl)
[c]Using Thr(Bzl)
[d]The Z protecting group was eliminated with TFA
[e]As a protected tripeptide purified by column chromatography
[f]Using Lys(Z)
[g]Using Arg(NO_2)
[h]Using Boc-Tca

Claims

1. A tripeptide of the formula (I):

X-Y-Pro-OH (I)

in which

X is Glp, Klc or Tca;

Y is Gly, Ala, Val, Ile, Pro, Abu, Nva, Nle, Ada, Cha, Phe, Trp, Tyr, Ser, Thr, Met, Gln, Lys, Arg or Leu,

or an acid addition salt or complex thereof.

2. A process for preparing a tripeptide of the formula (I) as defined in claim 1, or an acid addition salt or complex thereof, which comprises coupling with proline or a carboxyl-protected derivative thereof the amino acids X and Y or protected derivatives thereof by a conventional method of peptide chemistry and splitting off the protecting groups in a known manner from the tripeptides obtained in a protected form.

3. A process as claimed in claim 2, which comprises acylating proline methyl ester with the protected amino acid tert.-butyloxycarbonyl-Y-OH, saponifying the dipeptide methyl ester obtained, eliminating the tert.-butyloxycarbonyl protecting group, and acylating the dipeptide obtained or an acid

addition salt thereof with benzyloxycarbonylpyroglutamic acid pentafluorophenyl ester, and eliminating the protecting group from a protected tripeptide obtained.

4. A process as claimed in claim 3, wherein the dipeptide methyl ester is saponified with a dioxanic sodium hydroxide solution.

5. A process as claimed in claim 3 or 4, wherein the tert.-butyloxycarbonyl protecting group is eliminated by treatment with ethyl acetate.

6. A process as claimed in any of claims 3 to 5, wherein the protecting group is eliminated from the protected tripeptide by catalytic hydrogenation.

7. A pharmaceutical composition having anorexigenic activity, which comprises as an active ingredient a tripeptide of the formula (I) as defined in claim 1, or a pharmaceutically acceptable acid addition salt or complex thereof, in association with at least one conventional pharmaceutical carrier and/or excipient and/or further conventional additive.

8. A tripeptide of the formula (I) substantially as herein described in any of the Examples.

9. A process for preparing tripeptides of the formula (I) substantially as herein described in any of the examples.

10. A tripeptide of the formula (I) whenever prepared by a process as claimed in any of claims 2 to 6 and 9.

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